Comparative analysis of four *Zantedeschia* chloroplast genomes: expansion and contraction of the IR region, phylogenetic analyses and SSR genetic diversity assessment

Shuilian He [Equal first author, 1], **Yang Yang** [Equal first author, 2], **Ziwei Li** [1], **Yanbing Guo** [1], **Xuejiao Wang** [1], **Hongzhi Wu** [Corresp. 3]

[1] College of horticulture and landscape, Yunnan Agricuture University, Kunming, Yunnan, China

[2] College of Science, Yunnan Agricuture University, Kunming, Yunnan, China

[3] College of horticulture and landscape, Yunnan Agricultural University, Kunming, Yunnan, China

Corresponding Author: Hongzhi Wu
Email address: 1994061@ynau.edu.cn

The horticulturally important genus *Zantedeschia* (Araceae) comprises eight species of herbaceous perennials. We sequenced, assembled and analyzed the chloroplast (cp) genomes of four species of *Zantedeschia* (*Z. aethiopica*, *Z. odorata*, *Z. elliottiana*, and *Z. rehmannii*) to investigate the structure of the cp genome in the genus. According to our results, the cp genome of *Zantedeschia* ranges in size from 169,065 bp (*Z. aethiopica*) to 175,906 bp (*Z. elliottiana*). We identified a total of 112 unique genes, including 78 protein-coding genes, 30 transfer RNA (tRNA) genes and four ribosomal RNA (rRNA) genes. Comparison of our results with cp genomes from other species in the Araceae suggests that the relatively large sizes of the *Zantedeschia* cp genomes may result from IR expansion. The sampled *Zantedeschia* species formed a monophylogenetic clade in our phylogenetic analysis. Furthermore, the LSC and SSC regions in *Zantedeschia* are more divergent than the IR regions in the same genus, and non-coding regions showed generally higher divergence than coding regions. We identified a total of 410 cpSSR sites from the four *Zantedeschia* species studied. Genetic diversity analyses based on four polymorphic SSR markers from 134 cultivars of *Zantedeschia* suggested that high genetic diversity (I =0.934; He = 2.371) is present in the *Zantedeschia* cultivars. High genetic polymorphism from the cpSSR region suggests that cpSSR could be an effective tool for genetic diversity assessment and identification of *Zantedeschia* varieties.

INTRODUCTION

The genus *Zantedeschia* Spreng. (Trib. Richardieae, Araceae) had originally an entirely northeastern and southern African distribution. However, following introduction to Europe as ornamental plants in the seventeenth century, various species became widely naturalized across Europe, America, Oceania and Asia (Cruzcastillo et al. 2001). Two sections, *Zantedeschia* and *Aestivae*, are currently recognized in the genus *Zantedeschia*. Species in section *Zantedeschia*, *Z. aethiopica* and *Z. odorata* (Singh et al. 1996), can be recognized by the rhizomatous tuber and white flowers. Species in section *Aestivae,* however, have colorful (not white) flowers and discoid tubers (Singh et al. 1996; Wright & Burge 2000). Many attractive and colorful hybrids between species in section *Aestivae* have been artificially produced, the majority between *Z. albomaculata*, *Z. elliotiana, Z. rehmannii* and *Z. pentlandii* (Snijder et al. 2004a). *Zantedeschia* hybrids have subsequently become one of the most popular horticultural crops worldwide, in high demand as cut flowers, potted plants and flower baskets, as well as for use in flower beds. The genus *Zantedeschia* is also of horticultural interest, however, F1 hybrids between sections *Zantedeschia* and *Aestivae* are invariably albino.

Traditional and polyploidization breeding, as well as resistance to soft rot, have been the main focuses for previous research into the genus *Zantedeschia* (Snijder et al. 2004a; Snijder et al. 2004b; Wright & Triggs 2009; Wright & Burge 2000; Wright et al. 2002). A total of 43 novel EST-derived simple sequence repeat (SSR) markers have been identified in *Zantedeschia* by Wei et al (Wei et al. 2012), however, apart from this, the genetics and genomics of the genus *Zantedeschia*, which are of great importance in plant breeding, have received little research attention. We therefore recommend that further genomic resources from *Zantedeschia s*hould be developed as tools to assist molecular breeding research in this genus.

Our study focuses on four species of *Zantedeschia,* two from section *Zantedeschia* and two from section *Aestivae*. The aim of the study was to sequence, assemble and analyze the cp genome in *Zantedeschia*, to investigate any common characteristics or differences between the studied species and also to develop SSR markers in the *Zantedeschia* cp genome. Simple

sequence repeats (SSRs), or microsatellites, are short tandem repeats of two to more nucleotides in DNA sequences. The number of repeats is highly variable, whereas the regions of DNA flanking SSRs are highly conserved (Davierwala et al. 2000; Gur-Arie et al. 2000). SSR markers are polymerase chain reaction (PCR) -based, abundant, codominant, highly reproducible, and are distributed evenly across eukaryotic genomes (Powell et al. 1996). SSRs are widely used molecular markers to study genetic diversity, population structure, genetic mapping, phylogenetic studies, cultivar identification and marker-assisted selection. (Potter et al. 2015).

Photosysnthetic fixation of carbon in plants takes place in the chloroplasts, and is a primary function of these organelles. Chloroplasts have their own genome, as do mitochondria and it has been suggested that they were originally free-living cyanobacterium-like cells engulfed by ancient eukaryotic cells in an endosymbiotic relationship (Raven & Allen 2003). The cp genome is usually represented as a circular molecule, and has a conserved quadripartite structure comprising the small single copy (SSC) and large single copy (LSC) regions, separated by two copies of an inverted repeat (IR) region. Cp genomes have a highly conserved gene content, and most land plants have a nearly collinear gene sequence (Jansen et al. 2005).

Due to their lack of recombination, their compact size and their maternal inheritance (Birky 2001), cp genomes are considered to be useful DNA sequences for plant genetic diversity assessment, plant identification and phylogenetic studies.

We investigated the cp genomes from four species of *Zantedeschia.* Genomes were sequenced, assembled, annotated and mined for the presence of SSR markers using Illumina technology. We also made comparative sequence analysis studies of the cp sequences from our study species. These results are publicly available as a genetic resource for the study of *Zantedeschia* species, and it is our hope that they will provide a valuable resource for future genetic and phylogenetic studies into this important genus.

MATERIALS & METHODS

2.1 Plant material, DNA sequencing and cp genome assembly

Plant material of *Z. aethiopica* was collected from South Africa directly and has been planted in Kunming more than 30 years. *Z.odorata, Z. elliottiana, and Z. rehmannii* were collected from Netherlands. Total genomic DNA of the four species of *Zantedeschia* was extracted from the fresh leaves of tissue culture seedlings using a modified CTAB extraction protocol based on Doyle & Doyle (1987). Sequencing of the genomic DNA was performed using an Illumina Hiseq2000 (Illumina, CA, USA). Low quality reads were filtered out before *de novo* assembly of the cp genomes, and the resulting clean reads were assembled using the GetOrganelle pipeline (https://github.com/Kinggerm/ GetOrganelle). A reference genome *Colocasia esculenta* (JN105689) was used to check the contigs, using BLAST (https://blast.ncbi.nlm.nih.gov/), and the aligned contigs were then oriented according to the reference genome.

2.2 Gene Annotation and Sequence Analysis

The CpGAVAS pipeline (Liu et al. 2012) was used to annotate the genome and start/stop codons and intron/exon boundaries were adjusted in Geneious 8.1 (Kearse et al. 2012). The tRNA was identified using tRNAscan-SE v2.0 (Lowe & Chan 2016), and sequence data were subsequently deposited in GenBank. The online tool OGDraw v1.2 (http://ogdraw.mpimp-golm.mpg.de/) (Lohse et al. 2007) was used to generate a physical map of the genome.

2.3 Structure of Genome and Genome comparison

Pairwise sequence alignments of cp genomes were performed in MUMer (Kurtz et al. 2004). The complete cp genomes of the four species were then compared using mVISTA (Mayor et al. 2000) with the shuffle-LAGAN model Codon usage bias (RSCU) was calculated using MEGA v7.0 (Kumar et al. 2008).

Cp genome sequences of the four species were aligned using MAFFT (Katoh & Standley 2013) in Geneious 8.1(Kearse et al. 2012). Insertion/deletion polymorphisms (indels) were then identified using DnaSP version 5.1 with the cp genome of *Z. aethiopica* as a reference (Librado & Rozas 2009). Single nucleotide polymorphisms (SNPs), defined as variations in a single

nucleotide that occur at specific positions in the genome, were called using a custom Python script (https://www.biostars.org/p/119214/).

2.4 Phylogenetic analysis

Cp genome sequences of 11 species from Araceae and an outgroup (*Zea mays*, Poaceae) were downloaded from GenBank and an alignment with the four *Zantedeschia* cp genome sequences from our study was built using MAFFT (Katoh & Standley 2013) in Geneious 8.1(Kearse et al. 2012).

In order to investigate the phylogenetic placement of the genus *Zantedeschia* within the Araceae, a maximum likelihood tree was reconstructed in RaxML version 8.2.11 (Stamatakis 2014). Tree robustness was assessed using 1000 replicates of rapid four bootstrapping with the GTR+GAMMA substitution model.

2.5 Simple Sequence Repeats (SSRs)

SSR markers present in the *Zantedeschia* cp genome were found using Phobos v3.3.12 (Leese et al. 2008) and SSRHunter (Li & Wan 2005). Both these programs search for repeats using a recursive algorithm. We set the minimum number of repeats of mono-, di-, tri-, tetra-, penta-, and hexa-nucleutide repeats to 10, 5, 4, 3, 3 and 3 respectively. The inverted repeat region IRa was not considered in our SSR analysis.

We subsequently selected four SSRs motifs to investigate genetic diversity in *Zantedeschia*. A total of 134 cultivars from genus *Zantedeschia* were sampled. The experimented 134 cultivars include some local cultivars, but most of them are collected from Netherlands, the United States, New Zealand, and Taiwan for production and refreshed by Tissue culture every 3 years. Genetic diversity was investigated by calculating several indices: the number of alleles per locus (*Na*); the number of fffective alleles (*Ne*); Shannon's information index (*I*) and polymorphism information content (PIC). *Na*, *Ne*, I were calculated using GenALEx v. 6.4 (Peakall & Smouse 2006). PIC was calculated using PowerMarker 3.25 (Liu & Muse 2006).

RESULTS

3.1 Characteristics of Zantedeschia cp genomes

After assembly and annotation, the four *Zantedeschia* cp genomes obtained in this study were submitted to the NCBI database (accession numbers MH743153-5 and MG432242). The cp genomes of these *Zantedeschia* species ranged in length from169,065 bp (*Z. aethiopica*) to 175,906 bp (*Z. elliottiana*), and, as expected, the cp genomes of all four species were found to contain both the large and small single-copy regions, separated by a pair of inverted repeat regions (Figure 1& S1, Table 1). A total of 139 genes, of which 112 were unique, were identified, including 93 (78 unique) protein-coding genes, 38 (30 unique) transfer RNA (tRNA) genes and eight (four unique) ribosomal RNA (rRNA) genes (Table 2).

Interestingly, although the four species belong to a single genus, differences in gene construction can nevertheless be seen. *Z. elliottiana* had the largest number of genes (139). *Z. rehmannii* had 138 genes, differing from *Z. elliottiana* only in a single copy of rps19. *Z. odorata* had 134 genes, and differs from *Z. elliottiana* in having only single copies of *ndhE*, *ndhG*, *rps19*, *trnH-GUG* and *trnV-UAC*. Of all the species we studied, the cp genome of *Z. aethiopica* had the fewest of genes (131), and differed from *Z. elliottiana* in single copies of *ndhA*, *ndhE*, *ndhG*, *ndhH*, *ndhI*, *rps19*, *trnH-GUG* and *trnV-UAC* (Table 2).

The two species from section *Aestivae* (*Z. elliottiana*; *Z. rehmannii*) have larger cp genomes that those in section *Zantedeschia* (*Z. aethiopica* and *Z. odorata*), and the number of different protein-coding genes and tRNA genes is also higher in the cp genomes from plants in section *Aestivae*. Moreover, the size of the IR regions in species from section *Aestivae* was also larger than those in section *Zantedeschia*. Unlike the other studied species, which had no pseudogene, *Z. odorata* had two copies of the pseudogene *Ψycf68*.

The nucleotide composition of the *Zantedeschia* cp genome was asymmetric, with an overall GC content ranging from 35.3% to 35.6%, which is similar to other species in the Araceae (Tian et al. 2018). The largest GC content ratio was observed in the IR region (37.5%-

39.0%), and the smallest in the SSC region (28.2%-29.6%). All four of our study species showed the same trend (the GC content of the LSC and SSC regions was lower than that of the IR regions), which may be due to the tRNA genes and rRNA genes generally having fewer AT nucleotides (Chen et al. 2015; Meng et al. 2018; Zhou et al. 2017).

Introns are non-coding sequences within genes, and they play a very important role in the regulation of gene expression (Jiang et al. 2017). Introns are known to accumulate more mutations than functional genes, and because of this are used extensively in phylogenetic and population genetics studies (Xu 2003). We investigated 13 intron-containing genes in the species *Z. aethiopica*: 11 genes (*atpF*, *ndhA*, *ndhB*, *petB*, *petD*, *rpl16*, *rpl2*, *rpoC1*, *rps16*, *rps18*, *ycf68*) contained only one intron, while two genes (*clpP*, *ycf3*) contained two introns. *Z. elliottiana* and *Z. rehmannii* each had 12 intron-containing genes (similar to *Z. aethiopica* but lacking *clpP*), and 11 intron-containing genes were found in *Z. odorata* (as *Z. aethiopica* but lacking *rps18* and *ycf68*) (Table S1).

3.2 Genome comparison

An extremely important topic in genomics is the comparative analysis of cp genomes (Chen et al. 2012; Zhihai et al. 2016). We performed multiple alignments between the four cp genomes generated in this study to characterize their divergence. The alignments were conducted in mVISTA, using *Z. odorata* as a reference (Figure 2).

Unsurprisingly, we found that in our study species, the coding regions are more conserved than the non-coding regions. Furthermore, the LSC and SSC regions are more divergent from each other than are the IR regions. Intergenetic spacers (including *trnH-psbA*, *trnK-rps16*, *rps16-psbK*, *trnT-trnL*, *rbcL-psaL*, *clpP-psbB*, *ycf1-trnL*, *trnL-ndhB* in the LSC regions and *psaC-ndhE*, *rps15-ycf1*, *trnL-ycf2* in the IR regions) were found to be the most divergent regions of the four cp genomes. Of the coding regions, the greatest divergence was found in *clpP*, *rpl16, rps19*, *ycf1* and *ycf2*. This is in agreement with the results from previous studies (Park et al. 2017; Shen et al. 2017; Wu et al. 2017), and suggests that these regions might evolve rapidly in the genus *Zantedeschia*.

The level of sequence divergence in the aligned cp genome sequences from our four study species was explored using nucleotide variability (π), calculated using DnaSP version 5.1. The nucleotide variability (π) of these sequences was 0.0487, suggesting that the divergence between the cp genomes of these closely related species was relatively large. A total of 12,958 SNPs were found. We infer from these results that the *Zantedeschia* cp genome could be suitable for species-level phylogenetic analyses.

3.3 IR contraction and expansion in the Zantedeschia cp genome

A detailed comparison of the IRs of *Zantedeschia* cp genome was conducted and is presented in Figure 3. The cp genome sequences of 11 other species of Araceae downloaded from NCBI were included in our analysis in order to investigate changes in the IR sequence in *Zantedeschia*.

The IR regions of *Z. aethiopica, Z. odorata, Z. elliottiana* and *Z. rehmannii* had lengths of 32,331 bp, 36,549 bp, 39,445 bp, and 38,354 bp, respectively. The Even the shortest IR region of the four study species, that of *Z. aethiopica,* was longer than any of those from other species in the Araceae included in our study: *Colocasia esculenta* (25,273bp), *Pinellia ternata* (25,625bp), and *Dieffenbachia seguine* (25,235bp) (Tian et al. 2018). This expansion of the IR in the *Z. aethiopica* cp genome is because in this species, the rps15 gene has shifted from the SSC region to IRb at the SSC/IRb border, as well as to IRa at the SSC/Ira border. Other unusual, large expansions at the borders of IR regions have also been observed in our other three study species of *Zantedeschia*. In the two species *Z. elliottiana* and *Z. rehmannii,* the SSC/IRb border of occurs beside the *ndhE* gene, meaning that six genes (*ndhE*, *ndhG*, *ndhI*, *ndhA*, *ndhH*, *rps15*) have shifted from the SSC region to the IR region in these species. In the case of *Z. odorata*, the SSC/IRb border occurs beside the *ndhI* gene, and four genes (*ndhI*, *ndhA*, *ndhH*, and *rps15*) have therefore shifted from the SSC to the IR region. In all cases these shifts have resulted in a large expansion of the IR region.

3.4 Phylogenetic analysis

The cp genome sequences of 12 species (11 from the Araceae and the outgroup, *Zea mays*) were downloaded from NCBI, and the sequences were aligned together with the four *Zantedeschia* cp sequences from this study using Geneious 8.1 (Kearse et al. 2012). This alignment of the concatenated nucleotide sequences of a total of 16 cp genome sequences (an ingroup of 15 species from Araceae and the ougroup, *Zea mays*) was subjected to phylogenetic analyses. The phylogeny was reconstructed using maximum likelihood (ML), and the resulting phylogenetic tree was found to be in agreement with the traditional genus-level morphological taxonomy of the Araceae (Figure 4). Furthermore, the topology is consistent with the classical taxonomy of *Zantedeschia* at the genus level (Singh et al. 1996): Our four study species from the genus *Zantedeschia* formed a monophyletic clade with 100% bootstrap support. The morphologically defined sections *Zantedeschia* and *Aestivae* are also supported, with the two species from section *Zantedeschia* (*Z. odorata* and *Z. aethiopica*) sharing a more recent ancestor, and this clade forming a sister to the two species (*Z. elliottiana*, *Z. rehmannii*) from section *Aestivae*.

This is the first time the cp genomes of these four *Zantedeschia* species have been sequenced, and the sequences have enriched the phylogenetic research in Araceae and we believe that they will provide a useful resource for the further study of the genetic diversity in this family.

3.5 Simple Sequence Repeats (SSRs)

The SSR survey of the four *Zantedeschia* species in this study identified 73, 107, 110, and 120 potential SSRs motifs in the cp genome sequences of *Z. odorata* (175,906 bp), *Z. elliottiana* (175,067 bp); *Z. aethiopica* (169,065 bp), and *Z. rehmannii* (173,783 bp), respectively. The observed frequency of SSRs motifs was therefore approximately one SSR motif per 1,400-2,500 bp of cp genome (table S2). The majority of identified SSRs were mononucleotide repeats (*Z. elliottiana*: 52; *Z. rehmannii*: 55; *Z. aethiopica*: 61; *Z. odorata*: 54), followed by dinucleotide repeats (*Z. elliottiana*: 32; *Z. rehmannii*: 31; *Z. aethiopica*: 20; *Z. odorata*: 14). Most SSR repeats were AT-rich, and only 38 SSR repeats in *Zantedeschia* contained cytosine. These results are

consistent with previous findings that chloroplast SSRs usually consist of short polyA/T repeats (Nguyen et al. 2015). Most SSRs motifs were located in non-coding regions, in particular in the LSC region (70.0%), or in the IR regions (22.2%). Very few SSRs were located in the SSC region, and the ratio was less than 0.08%. A similar result has been observed in other studies, suggesting that the cp genome LSC region always contains high ratio of SSR motifs (Chi et al. 2018; Jian et al. 2018).

Four tri-SSRs motifs (Table 3) were used to investigate genetic diversity in *Zantedeschia*. We sampled a total of 134 cultivars. All four cp SSR loci studied were polymorphic in the genus *Zantedeschia*. The number of alleles (*Na*) of the genus was 3.000, the number of effective alleles (*Ne*) was 2.371, the Shannon's information index (*I*) was 0.934 and the polymorphism information content (PIC) was 0.388 (Table 4).

These results suggest that chloroplast SSR markers could be useful tools to study genetic diversity in *Zantedeschia*, and furthermore that this could be an effective method to select germplasm for the improvement of ornamental cultivars in *Zantedeschia.*

DISCUSSION

IR contraction and expansion in the cp genome of the genus Zantedeschia

With the exception of certain plants in the Fabaceae, and all conifers, the cp genomes of most plants display large inverted repeats (IRs) (Aii et al. 1997). It has been suggested that these IRs have important roles in conserving essential genes and stabilizing the structure of chloroplast DNA (Palmer & Thompson 1982). Most plant species have an IR of about 25 kbp in size (Aii et al. 1997), and while the sequences of IRs are generally conserved, contraction and expansion events at the borders of these regions are common. During land plant evolution, there have been multiple instances of IR expansion or contraction that have involved the shifting of complete genes from the SSC regions into the IR or *vice versa*, resulting in the IR in land plants varying in size from 10 to 76 kbp. These events change the boundaries of the IR regions with the LSC or SSC regions, explaining the variation in size of the cp genome (Raubeson et al. 2007; Xia et al.

2009; Yao et al. 2015). The terminal IR gene, which is adjacent to the SSC region, is highly conserved across most land plants, and in most species, including those in the genera *Rosa*, *Lancea* and *Paeonia* (Meng et al. 2018), *trnN-GUU* is the last full-length IR gene at the IR/SSC boundary. This is strong evidence that this was an ancestral IR/SSC endpoint that has been retained in most lineages (Zhu et al. 2016).

When we compared the cp genome IR boundary in various genera in the Araceae (*Lemna*, *Symplocarpus*, *Wolffia*, *Acorus*, *Symplocarpus*, *Pinellia*, *Colocasia* and *Dieffenbachia*), we found in this family, the IR generally terminates at the *trnN-GUU* gene at the IR/SSC boundary (Figure 3) like most land plants. Howerver, we did find several minor IR extensions into the SSC in the Araceae genera *Acorus*, *Wolffiella*, *Spirodela*, *Lemna* as well as in *Z. aethiopica*. Minor IR extensions into the LSC region have occasionally occurred, as in *Acorus americanus*. Surprisingly, large expansions have occurred in three species of *Zantedeschia*, and in particular in *Z. elliottiana,* in which six genes of the SSC region and two genes of the LSC region have shifted into the IR region. The variation in the size of the IR region may not only explain the differences in length between different *Zantedeschia* cp genomes, but may also affect the rates of substitution and of plastome sequence evolution. Indeed, there is evidence that the IR has significant influence on the rates of evolution of plastid genomes, and the IR has been demonstrated to have lower rates of substitution (non-coding as well as synonymous and nonsynonymous) than do single-copy genes in several groups of angiosperms including carnivorous plants (Kim et al. 2009; Susann et al. 2013; Wolfe et al. 1987; Yi et al. 2012) as well as in some gymnosperms, such as *Cycas* (Wu & Chaw 2015). However, in plants totally lacking the IR, such as the legume clade, those genes which in other groups are IR genes have a synonymous substitution rate similar to that of single-copy genes (Perry & Wolfe 2002). Wolfe (1987) and Perry & Wolfe (2002) suggest that a copy-dependent repair mechanism, such as gene conversion, would explain the lower rate of substitutions seen in the IR. Gene conversion has been demonstrated in plastids (Khakhlova & Bock 2006), and is suggested to have been responsible for small increases and decreases in the size of the IR region (Goulding et al. 1996).

SSR genetic diversity in Zantedeschia

Genetic diversity assessment is used to characterize germplasm and also has a role in conservation, allowing the identification of potential parents for breeding programs (Friedt et al. 2007). Genetic diversity in germplasm collections is commonly assessed through the use of molecular markers. Inter-sample sequence repeats (ISSRs), amplified fragment length polymorphisms (AFLPs), and random amplified polymorphic DNA (RAPD) markers have allowed the development of DNA fingerprinting for the identification of cultivars of *Zantedeschia* (Bo et al. 2012; Hamada & Hagimori 1996), revealing levels of genetic variation (Zhang 2009; Zhen & XU 2013). However, as well as being labor-intensive and having only unstable reproducibility, a major weakness of these molecular markers is that they are dominant markers, and cannot therefore distinguish heterozygous and homozygous genotypes (Tan et al. 2012). Simple sequence repeat (SSR) markers possess several advantages over the other molecular markers, including co-dominance, high polymorphism, and good reproducibility (Morgante et al. 2002). Furthermore, SSRs from chloroplast DNA are powerful tools in evolutionary and population genetics (Dong et al. 2013; Dong et al. 2016; Flannery et al. 2006; Suo et al. 2016) for the construction of linkage maps and to inform the breeding of crop plants (Powell et al. 1995; Xue et al. 2012), because they are uniparentally inherited and can be highly variable even intraspecifically.

Wei et al. (2012) developed 43 polymorphic SSRs loci from expressed sequence tags (ESTs) from white calla lily (section *Zantedeschia*) . Moderately high levels of genetic diversity were reported from analyses of 24 wild or cultivated accessions of white calla lily. The observed/expected heterozygosity (H_O/H_E) was 0.501/0.662, respectively, and the mean number of alleles per locus (*Na*) was 5.23. The PIC was found to be 0.446 (Wei et al. 2012). In a subsequent study into the genetic diversity of the colored calla lily (section *Aestivae*) using 31 EST-SSRs, Wei et al. (2017) showed that: $Na = 3.58$; $H_O = 0.453$; $H_E = 0.478$, PIC = 0.26 and $Ne = 2.18$. Although the two studies both used EST-SSRs, evaluation of the genetic diversity revealed slight differences.

Our present study is the first to develop and employ SSR markers from the cp genome of genus *Zantedeschia*. In order to utilize these markers for the identification of cultivars, we choose four representative polymorphic tri-SSR markers and used these to assess genetic diversity in 134 cultivars of *Zantedeschia.* Compared with EST-SSRs diversity analyses from previous studies, our results show a low level of genetic diversity in *Zantedeschia*, with *Na*= 3.00, *Ne* = 2.371, and PIC = 0.388. Furthermore, cpSSRs showed lower diversity than the nSSRs. Similar results have been reported from other species using both types of SSR markers (Pakkad et al. 2008; Robledo-Arnuncio & Gil 2005; Setsuko et al. 2007), and reflects the low substitution rate in plant cpDNA sequences compared with that in nDNA (Wolfe et al. 1987). SSRs from mitochondrial or cp genomes have been developed in many species and have been used for the analysis of genetic diversity (Song et al. 2014; Wheeler et al. 2014), however this study represents the first time an organic SSR system has been developed in Araceae. SSRs developed from the *Zantedeschia* uniparentally inherited and non-recombinant cp genome also have the advantages of nuclear SSRs, and we believe that they will be useful for genetic analysis in this horticulturally important genus.

CONCLUSIONS

This study presents the sequenced cp genome sequences from four horticulturally important species of *Zantedeschia* (Araceae), a genus native to northeastern and southern Africa and now globally naturalized. The sequencing, assembly, annotation and comparative analyses revealed that the cp genome of *Zantedeschia* has a quadruple structure, with a gene order and GC content similar to those of typical angiosperm cp genomes. However, unusual IR expansion was found in this genus. SSR genetic diversity assessment showed that *Zantedeschia* has moderately high-level diversity. Phylogenetic analysis showed that the sampled species of the genus *Zantedeschia* formed a monophyletic clade. These sequences will enable us to assess genome-wide mutational dynamics within the family Araceae, and moreover, will facilitate investigations into gene expression and genetic variation within these ornamental species.

ACKNOWLEDGEMENTS

We thank Dr. Andan Zhu and Dr. Shudong Zhang from the Kunming Institute of Botany, Chinese Academy of Sciences for their help in the revision of the manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Supplementary Materials: Supplementary materials are available online.

Author Contributions:

- Shuilian He and Hongzhi Wu conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Ziwei Li, Yang Yang Yanbing Guo and Xuejiao Wang conceived and designed the experiments, contributed analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

REFERENCES

Aii J, Kishima Y, Mikami T, and Adachi T. 1997. Expansion of the IR in the chloroplast genomes of buckwheat species is due to incorporation of an SSC sequence that could be mediated by an inversion. *Current Genetics* 31:276-279.

Birky CW. 2001. The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. *Annual Review of Genetics* 35:125.

Bo LU, Zheng YH, Peng F, Shu XC, and Chen XX. 2012. Optimization of RAPD reaction system by uniform design on *Zantedeschia* hybrida. *Northern Horticulture*:123-126.

Chen S, Xu J, Liu C, Zhu Y, Nelson DR, Zhou S, Li C, Wang L, Guo X, and Sun Y. 2012. Genome sequence of the model medicinal mushroom *Ganoderma lucidum*. *Nature Communications* 3:913.

Chen X, Li Q, Li Y, Qian J, and Han J. 2015. Chloroplast genome of *Aconitum barbatum* var. *puberulum* (Ranunculaceae) derived from CCS reads using the PacBio RS platform. *Frontiers in Plant Science* 6:42.

Chi XF, Wang JL, Gao QB, Zhang FQ, and Chen SL. 2018. The complete chloroplast genomes of two *Lancea* species with comparative analysis. *Molecules* 23:602.

346 Cruzcastillo JG, Mendozaramirez J, and Torreslima PA. 2001. Shade, fertilizers and a natural
347 bioregulator to improve *Zantedeschia* growth in a Mexican tropical upland area. *Journal*
348 *of agriculture of the University of Puerto Rico* 85:135-142.
349 Davierwala AP, Ramakrishna W, Ranjekar PK, and Gupta VS. 2000. Sequence variations at a
350 complex microsatellite locus in rice and its conservation in cereals. *Theoretical and*
351 *Applied Genetics* 101:1291-1298.
352 Dong W, Chao X, Tao C, Lin K, and Zhou S. 2013. Sequencing angiosperm plastid genomes
353 made easy: A Complete set of universal primers and a case study on the phylogeny of
354 Saxifragales. *Genome Biology and Evolution* 5:989.
355 Dong W, Xu C, Li D, Jin X, Li R, Lu Q, and Suo Z. 2016. Comparative analysis of the complete
356 chloroplast genome sequences in psammophytic *Haloxylon* species (Amaranthaceae).
357 *Peerj* 4:e2699.
358 Doyle JJ, and Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf
359 tissue. *Phytochemical Bull* 19:11-15.
360 Flannery ML, Mitchell FJG, Coyne S, Kavanagh TA, Burke JI, Salamin N, Dowding P, and
361 Hodkinson TR. 2006. Plastid genome characterisation in *Brassica* and Brassicaceae using
362 a new set of nine SSRs. *Theoretical and Applied Genetics* 113:1221-1231.
363 Friedt W, Snowdon R, Ordon F, and Ahlemeyer J. 2007. Plant Breeding: assessment of genetic
364 diversity in crop plants and its exploitation in breeding. *Progress in Botany*: Springer,
365 Berlin, Heidelberg, 151-178.
366 Goulding SE, Wolfe KH, Olmstead RG, and Morden CW. 1996. Ebb and flow of the chloroplast
367 inverted repeat. *Molecular & General Genetics Mgg* 252:195-206.
368 Gur-Arie R, Cohen CJ, Eitan Y, Shelef L, and Kashi Y. 2000. Simple sequence repeats in
369 *Escherichia coli*: abundance, distribution, composition, and polymorphism. *Genome*
370 *Research* 10:62-71.
371 Hamada K, and Hagimori M. 1996. RAPD-based method for cultivar-identification of calla lily
372 (*Zantedeschia* spp.). *Scientia Horticulturae* 65:215-218.

373 Jansen RK, Raubeson LA, Boore JL, Depamphilis CW, Chumley TW, Haberle RC, Wyman SK,
374 Alverson AJ, Peery R, and Herman SJ. 2005. Methods for obtaining and analyzing whole
375 chloroplast genome sequences. *Methods in Enzymology* 395:348.
376 Jian HY, Zhang YH, Yan HJ, Qiu XQ, Wang QG, Li SB, and Zhang SD. 2018. The complete
377 chloroplast genome of a key ancestor of modern roses, *Rosa chinensis* var. *spontanea*,
378 and a comparison with congeneric species. *Molecules* 23:389.
379 Jiang X, Yang C, Baosheng L, Shuiming X, Qinggang Y, Rui B, He S, Linlin D, Xiwen L, and
380 Jun Q. 2017. Panax ginseng genome examination for ginsenoside biosynthesis.
381 *Gigascience* 6:1-15.
382 Katoh K, and Standley DM. 2013. MAFFT multiple sequence alignment software version 7:
383 improvements in performance and usability. *Molecular Biology and Evolution* 30:772.
384 Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A,
385 Markowitz S, and Duran C. 2012. Geneious Basic: An integrated and extendable desktop
386 software platform for the organization and analysis of sequence data. *Bioinformatics*
387 28:1647.
388 Khakhlova O, and Bock R. 2006. Elimination of deleterious mutations in plastid genomes by
389 gene conversion. *Plant Journal* 46:85-94.
390 Kim YK, Park C-w, and Kim K-J. 2009. Complete chloroplast DNA sequence from a Korean
391 endemic genus, *Megaleranthis saniculifolia*, and its evolutionary implications. *Molecules*
392 *and Cells* 27:365-381.
393 Kumar S, Nei M, Dudley J, and Tamura K. 2008. MEGA: a biologist-centric software for
394 evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9:299-
395 306.
396 Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, and Salzberg SL. 2004.
397 Versatile and open software for comparing large genomes. *Genome Biology* 5:R12.
398 Leese F, Mayer C, and Held C. 2008. Isolation of microsatellites from unknown genomes using
399 known genomes as enrichment templates. *Limnology & Oceanography Methods* 6:412-

426.

Li Q, and Wan JM. 2005. SSRHunter: development of a local searching software for SSR sites. *Hereditas* 27:808.

Librado P, and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451-1452.

Liu C, Shi L, Zhu Y, Chen H, Zhang J, Lin X, and Guan X. 2012. CpGAVAS, an integrated web server for the annotation, visualization, analysis, and GenBank submission of completely sequenced chloroplast genome sequences. *BMC Genomics* 13:715.

Liu K, and Muse SV. 2006. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21:2128-2129.

Lohse M, Drechsel O, and Bock R. 2007. OrganellarGenomeDRAW (OGDRAW): a tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. *Current Genetics* 52:267-274.

Lowe TM, and Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Research* 44:W54-W57.

Mayor C, Brudno M, Schwartz JR, Poliakov A, Rubin EM, Frazer KA, Pachter LS, and Dubchak I. 2000. VISTA : visualizing global DNA sequence alignments of arbitrary length. *Bioinformatics* 16:1046-1047.

Meng J, Li XP, Li HT, Yang JB, Wang H, and He J. 2018. Comparative analysis of the complete chloroplast genomes of four *Aconitum* medicinal species. *Molecules* 23:1015.

Morgante M, Hanafey M, and Powell W. 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics* 30:194-200.

Nguyen PAT, Kim JS, and Kim JH. 2015. The complete chloroplast genome of colchicine plants (*Colchicum autumnale* L. and *Gloriosa superba* L.) and its application for identifying the genus. *Planta* 242:223-237.

Pakkad G, Ueno S, and Yoshimaru H. 2008. Genetic diversity and differentiation of *Quercus semiserrata* Roxb. in northern Thailand revealed by nuclear and chloroplast

427 microsatellite markers. *Forest Ecology and Management* 255:1067-1077.
428 Palmer JD, and Thompson WF. 1982. Chloroplast DNA rearrangements are more frequent when
429 a large inverted repeat sequence is lost. *Cell* 29:537-550.
430 Park I, Kim WJ, Yeo SM, Choi G, Kang YM, Piao R, and Moon BC. 2017. The complete
431 chloroplast genome sequences of *Fritillaria ussuriensis* Maxim. and *Fritillaria cirrhosa*
432 D. Don, and comparative analysis with other *Fritillaria* species. *Molecules* 22:982.
433 Peakall R, and Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic
434 software for teaching and research. *Molecular Ecology Notes* 6:288-295.
435 Perry AS, and Wolfe KH. 2002. Nucleotide Substitution Rates in Legume Chloroplast DNA
436 Depend on the Presence of the Inverted Repeat. *Journal of Molecular Evolution* 55:501-
437 508.
438 Potter KM, Hipkins VD, Mahalovich MF, and Means RE. 2015. Nuclear genetic variation across
439 the range of Ponderosa pine (*Pinus ponderosa*): Phylogeographic, taxonomic and
440 conservation implications. *Tree Genetics & Genomes* 11:38.
441 Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, and Rafalski A. 1996. The
442 comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm
443 analysis. *Molecular Breeding* 2:225-238.
444 Powell W, Morgante M, Mcdevitt R, Vendramin GG, and Rafalski JA. 1995. Polymorphic
445 simple sequence repeat regions in chloroplast genomes: applications to the population
446 genetics of pines. *Proceedings of the National Academy of Sciences of the United States*
447 *of America* 92:7759.
448 Raubeson LA, Peery R, Chumley TW, Dziubek C, Fourcade HM, Boore JL, and Jansen RK.
449 2007. Comparative chloroplast genomics: analyses including new sequences from the
450 angiosperms *Nuphar advena* and *Ranunculus macranthus*. *BMC Genomics* 8:174.
451 Raven JA, and Allen JF. 2003. Genomics and chloroplast evolution: what did cyanobacteria do
452 for plants? *Genome Biology* 4:1-5.
453 Robledo-Arnuncio JJ, and Gil L. 2005. Patterns of pollen dispersal in a small population of *Pinus*

sylvestris L. revealed by total-exclusion paternity analysis. *Heredity* 94:13-22.

Setsuko S, Ishida K, Ueno S, Tsumura Y, and Tomaru N. 2007. Population differentiation and gene flow within a metapopulation of a threatened tree, *Magnolia stellata* (Magnoliaceae). *American Journal of Botany* 94:128-136.

Shen XF, Wu ML, Liao BS, Liu ZX, Bai R, Xiao SM, Li XW, Zhang BL, Xu J, and Chen SL. 2017. Complete chloroplast genome sequence and phylogenetic analysis of the medicinal plant *Artemisia annua*. *Molecules* 22:1330.

Singh Y, Wyk AEV, and Baijnath H. 1996. Taxonomic notes on the genus *Zantedeschia* Spreng. (Araceae) in southern Africa. *South African Journal of Botany* 62:321-324.

Snijder RC, Cho H-R, Hendriks MM, Lindhout P, and van Tuyl JM. 2004a. Genetic variation in *Zantedeschia* spp.(Araceae) for resistance to soft rot caused by *Erwinia carotovora* subsp. *carotovora*. *Euphytica* 135:119-128.

Snijder RC, Lindhout P, and van Tuyl JM. 2004b. Genetic control of resistance to soft rot caused by *Erwinia carotovora* subsp. *carotovora* in *Zantedeschia* spp. (Araceae), section *Aestivae*. *Euphytica* 136:319-325.

Song SL, Lim PE, Phang SM, Lee WW, Dang DH, and Prathep A. 2014. Development of chloroplast simple sequence repeats (cpSSRs) for the intraspecific study of *Gracilaria tenuistipitata* (Gracilariales, Rhodophyta) from different populations. *BMC Research Notes* 7:77-77.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312.

Suo Z, Li WY, Jin XB, and Zhang HJ. 2016. A new nuclear DNA marker revealing both microsatellite variations and single nucleotide polymorphic loci: A case study on classification of cultivars in *Lagerstroemia indica* L. *Journal of Microbial & Biochemical Technology* 8:266-271.

Susann W, Bastian S, W. dC, and Müller KF. 2013. Disproportional plastome-wide increase of substitution rates and relaxed purifying selection in genes of carnivorous Lentibulariaceae.

Molecular Biology and Evolution:3.

Tan C, Wu Y, Anderson MP, Tauer C, and Samuels T. 2012. Development of simple sequence repeat markers for bermuda grass from its expressed sequence tag sequences and preexisting sorghum SSR markers. *Molecular Breeding* 29:23-30.

Tian N, Han LM, Chen C, and Wang ZZ. 2018. The complete chloroplast genome sequence of *Epipremnum aureum* and its comparative analysis among eight Araceae species. *PloS one* 13:e0192956.

Wei Z-Z, Luo L-B, Zhang H-L, Xiong M, Wang X, and Zhou D. 2012. Identification and characterization of 43 novel polymorphic EST-SSR markers for arum lily, *Zantedeschia aethiopica* (Araceae). *American Journal of Botany* 99:e493-e497.

Wei Z, Zhang H, Wang Y, Li Y, Xiong M, Wang X, and Zhou D. 2017. Assessing genetic diversity and population differentiation of colored calla lily (*Zantedeschia* Hybrid) for an efficient breeding program. *Genes* 8:168.

Wheeler GL, Dorman HE, Alenda B, Lavanya C, and Wallace LE. 2014. A review of the prevalence, utility, and caveats of using chloroplast simple sequence repeats for studies of plant biology. *Applications in Plant Sciences* 2:1400059.

Wolfe KH, Li WH, and Sharp PM. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences of the United States of America* 84:9054-9058.

Wright P, and Triggs C. 2009. Factors affecting bacterial soft rot of *Zantedeschia* tubers. *New Zealand Journal of Crop and Horticultural Science* 37:345-350.

Wright PJ, and Burge GK. 2000. Irrigation, sawdust mulch, and Enhance® biocide affects soft rot incidence, and flower and tuber production of calla. *New Zealand Journal of Crop and Horticultural Science* 28:225-231.

Wright PJ, Burge GK, and Triggs CM. 2002. Effects of cessation of irrigation and time of lifting of tubers on bacterial soft rot of calla (*Zantedeschia* spp.) tubers. *New Zealand Journal of Crop and Horticultural Science* 30:265-272.

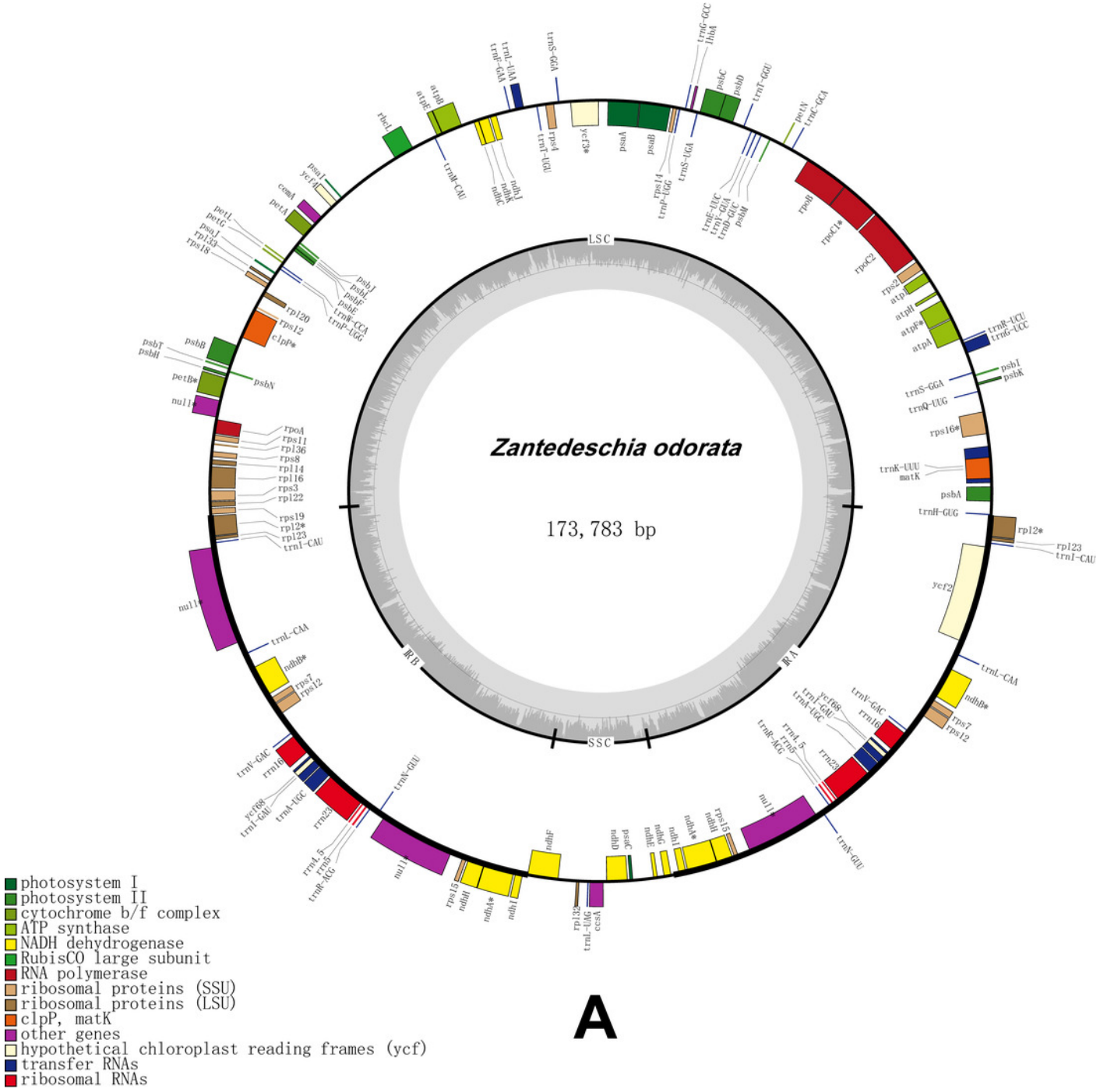
508 Wu CS, and Chaw SM. 2015. Evolutionary stasis in cycad plastomes and the first case of
509 plastome GC-biased gene conversion. *Genome Biology and Evolution* 7:2000-2009.
510 Wu ML, Li Q, Hu ZG, Li XW, and Chen SL. 2017. The complete *Amomum kravanh* chloroplast
511 genome sequence and phylogenetic analysis of the *Commelinids*. *Molecules* 22:1875.
512 Xia Z, Wang YZ, and Smith JF. 2009. Familial placement and relations of *Rehmannia a*nd
513 *Triaenophora* (Scrophulariaceae s.l.) inferred from five gene regions. *American Journal*
514 *of Botany* 96:519-530.
515 Xu JW. 2003. The first intron of rice EPSP synthase enhances expression of foreign gene.
516 *Science in China Ser C* 46:561.
517 Xue J, Wang S, and Zhou SI. 2012. Polymorphic chloroplast microsatellite loci in *Nelumbo*
518 (Nelumbonaceae). *American Journal of Botany* 99:240-244.
519 Yao X, Tang P, Li Z, Li D, Liu Y, and Huang H. 2015. The first complete chloroplast genome
520 sequences in Actinidiaceae: Genome structure and comparative analysis. *PloS one*
521 10:e0129347.
522 Yi DK, Lee H-L, Sun B-Y, Chung MY, and Kim K-J. 2012. The complete chloroplast DNA
523 sequence of *Eleutherococcus senticosus*(Araliaceae); Comparative evolutionary analyses
524 with other three asterids. *Molecules and Cells* 33:497-508.
525 Zhang Y. 2009. Optimization of ISSR reaction system and preliminary study on *Zantedeschia*.
526 *Molecular Plant Breeding*:827-832.
527 Zhen C, and XU. 2013. Physiological and biochemical and resistance changes and Issr
528 polymorphic analysis exposed to ~(12)C~(6+) heavy ion radiation on calla lily. *Journal*
529 *of Nuclear Agricultural Sciences* 27:552-556.
530 Zhihai H, Jiang X, Shuiming X, Baosheng L, Yuan G, Chaochao Z, Xiaohui Q, Wen X, and
531 Shilin C. 2016. Comparative optical genome analysis of two pangolin species: *Manis*
532 *pentadactyla* and *Manis javanica*. *Gigascience* 5:1-5.
533 Zhou J, Chen X, Cui Y, Sun W, Li Y, Wang Y, Song J, and Yao H. 2017. Molecular structure
534 and phylogenetic analyses of complete chloroplast genomes of two *Aristolochia*

medicinal species. *International Journal of Molecular Sciences* 18:1839.

Zhu A, Guo W, Gupta S, Fan W, and Mower JP. 2016. Evolutionary dynamics of the plastid inverted repeat: the effects of expansion, contraction, and loss on substitution rates. *New Phytologist* 209:1747-1756.

Figure 1

Gene map of chloroplast genome of *Z. odorata* .



A

Figure 2

Comparison of four cp genomes using the mVISTA alignment program.

The x-axis represents the coordinates in the cp genome. The y-axis indicated the average percent identity of sequence similarity in the aligned regions, ranging between 50% and 100%, Purple bars represent exons, blue bars represent untranslated regions (UTRs), pink bars represent noncoding sequences (CNS), gray bars represent mRNA, and white bars represent differences of genomics.

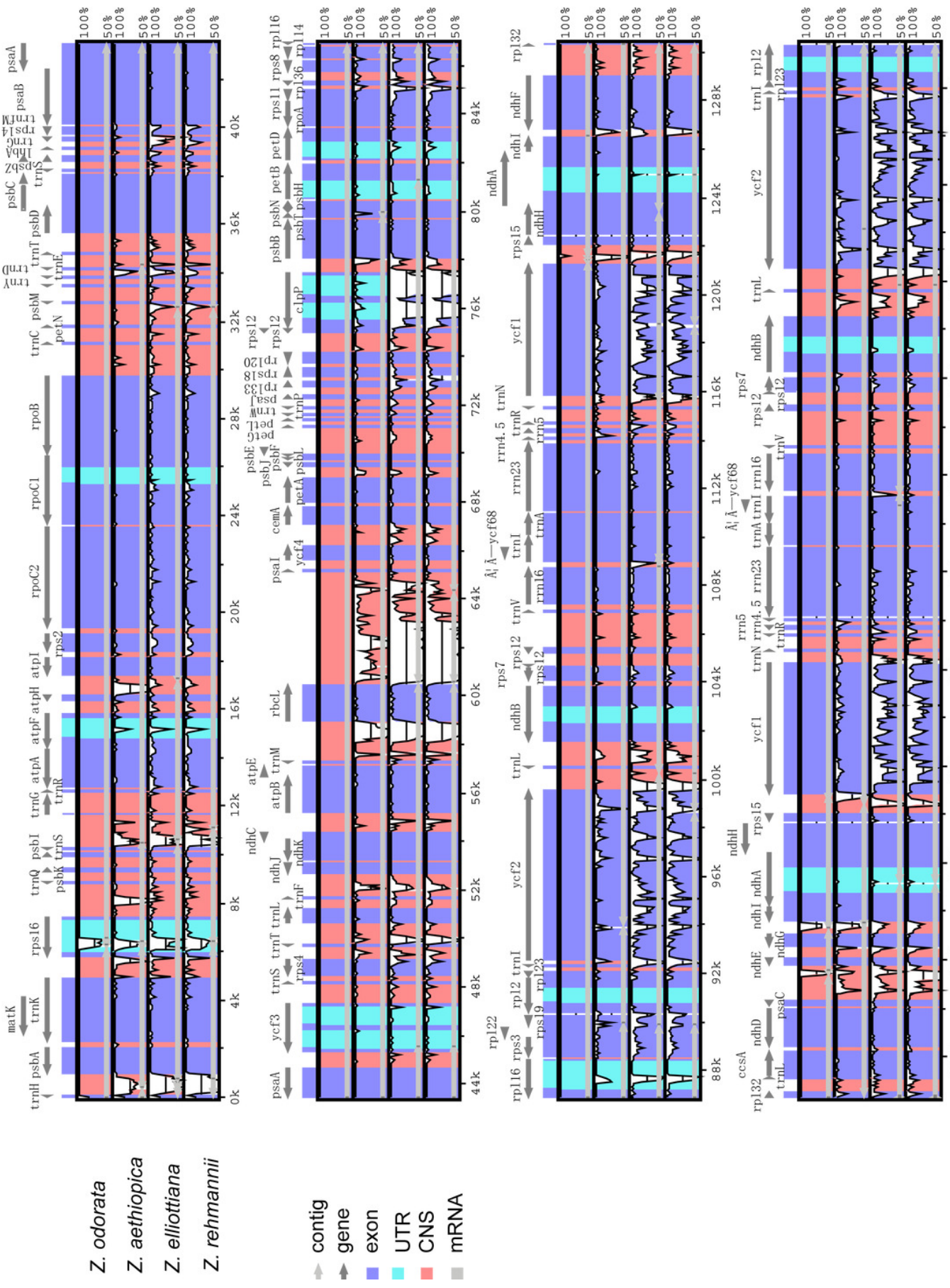

Z. odorata
Z. aethiopica
Z. elliottiana
Z. rehmannii
contig
gene
exon
UTR
CNS
mRNA

Figure 3

Genes of IR region in Araceae (Genes which are only partially duplicated in the IR are not shown).

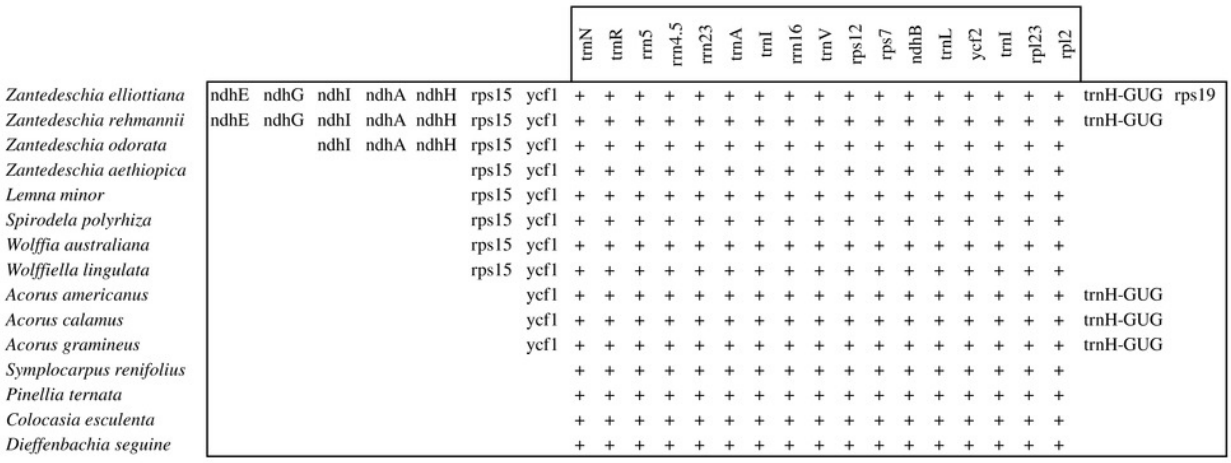


Figure 4

The maximum likelihood (ML) phylogenetic tree based on 14 complete chloroplast genome sequence.

Numbers at the right of nodes are bootstrap support values.

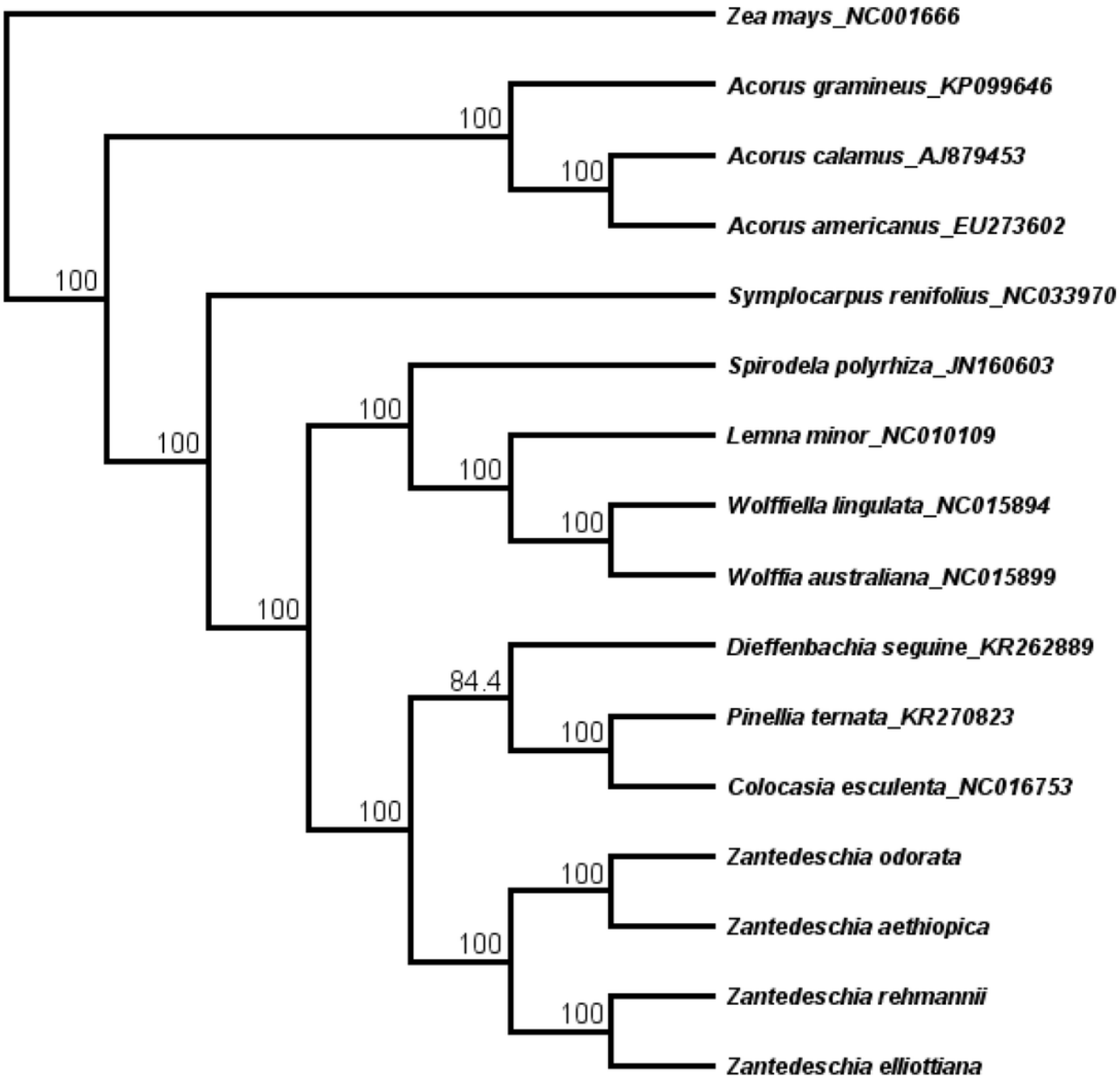

Zea mays_NC001666
Acorus gramineus_KP099646
Acorus calamus_AJ879453
Acorus americanus_EU273602
Symplocarpus renifolius_NC033970
Spirodela polyrhiza_JN160603
Lemna minor_NC010109
Wolffiella lingulata_NC015894
Wolffia australiana_NC015899
Dieffenbachia seguine_KR262889
Pinellia ternata_KR270823
Colocasia esculenta_NC016753
Zantedeschia odorata
Zantedeschia aethiopica
Zantedeschia rehmannii
Zantedeschia elliottiana
100
100
100
100
100
100
100
100
84.4
100
100
100
100

Table 1(on next page)

The basic characteristics of chloroplast genomes of four *Zantedeschia* species

Table 1. The basic characteristics of chloroplast genomes of four *Zantedeschia* species.

Characteristics	***Z. elliottiana***	***Z. rehmannii***	***Z. aethiopica***	***Z. odorata***
GenBank numbers	*MH743153*	*MH743154*	*MH743155*	*MG432242*
Total cp genome size/bp	175,906	175,067	169,065	173,783
LSC size/bp	88,584	90,020	89,695	90,322
IR size /bp	39,445	38,354	32,331	36,549
SSC size /bp	8,432	8,338	14,715	10,363
Total number of genes	139	138	131	134
Number of different protein-coding genes	93	92	87	90
Number of different tRNA genes	38	38	37	36
Number of different rRNA genes	8	8	8	8
Number of gene in IR region	54	52	40	46
Number of pseudogene	0	0	0	2
GC content (%)	35.4	35.6	35.6	35.3
GC content of LSC (%)	34.2	34.4	34.1	33.7
GC content of IR (%)	37.5	37.7	39.0	38.2
GC content of SSC (%)	28.7	29.1	29.6	28.2

Table 2(on next page)

Genes present in the *Zantedeschia* chloroplast genome

[2]Two gene copies in IRs. [%]shows only one copy in *Z. rehmannii*, [@]shows only one copy in *Z. aethiopica*, [&]shows only one copy in *Z. odorata*, [#]shows gene not exist in *Z. aethiopica*, *shows gene not exist in *Z. odorata*. *Ψ* shows pseudogenes.

Table 2. Genes present in the *Zantedeschia* chloroplast genome.

Category	Gene groups	Name of genes
Self-replication	Large subunit of ribosomal proteins	*rpl2*[2], *rpl14, rpl16,rpl20, rpl22, rpl23*[2], *rpl32, rpl33, rpl36*
	Small subunit of ribosomal proteins	*rps2, rps3, rps4, rps7*[2], *rps8, rps11, rps12*[2], *rps14, rps15*[2], *rps16, rps18, rps19*[2@&%]
	DNA dependent RNA polymerase	*rpoA, rpoB, rpoC1, rpoC2*
	Ribosomal RNA genes	*rrn4.5*[2], *rrn5*[2], *rrn16*[2], *rrn23*[2]
	Transfer RNA genes	*trnA(UGC)*[2], *trnC(GCA)*, *trnD(GUC)*, *trnE(UUC)*, *trnF(GAA)*, *trnfM(CAU)*, *trnG(GCC)*, *trnG(UCC)*, *trnH(GUG)*[2@&], *trnI(CAU)*[2], *trnI(GAU)*[2], *trnK(UUU)*, *trnL(CAA)*[2], *trnL(UAA)*, *trnL(UAG)*, *trnM(CAU)*, *trnN(GUU)*[2] , *trnP(UGG)*, *trnQ(UUG)*, *trnR(ACG)*[2], *trnR(UCU)*, *trnS(GCU)*, *trnS(GGA)*, *trnS(UGA)*, *trnT(GGU)*, *trnT(UGU)*, *trnV(GAC)*[2], *trnV(UAC)*[*#,] *trnW(CCA)*, *trnY(GUA)*
Photosynthesis	NADH oxidoreductase	*ndhA*[2@], *ndhB*[2], *ndhC, ndhD, ndhE*[2@&], *ndhF, ndhG*[2@&], *ndhH*[2@], *ndhI*[2@], *ndhJ, ndhK*
	Photosystem I	*psaA, psaB, psaC, psaI, psaJ,ycf3, ycf4*
	Photosystem II	*psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ*
	Cytochrome b/f complex	*petA, petB, petD, petG, petL, petN*
	ATP synthase	*atpA, atpB, atpE, atpF, atpH, atpI*
	RubisCo large subunit	*rbcL*
Other genes	Maturase K	*matK,cemA*
	C-type cytochrome synthesis gene	*ccsA*
	Protease	*clpP*
	Proteins of unknown function	*ycf1*[2], *ycf2*[2], *ycf68*[2*]
	pseudogene	*Ψycf68*[2] (in *Z. odorata*)

[2]Two gene copies in IRs. [%]shows only one copy in *Z. rehmannii*, [@]shows only one copy in *Z. aethiopica*, [&]shows only one copy in *Z. odorata*, [#]shows gene not exist in *Z. aethiopica*, *shows gene not exist in *Z. odorata*. *Ψ* shows pseudogenes.

Table 3(on next page)

Characteristics of the four SSR motifs for *Z. odorata*. Forward and reverse primer sequences, Annealing temperature (Tm), repeat motifs.

Table 3. Characteristics of the four SSR motifs for *Z. odorata*. Forward and reverse primer sequences, Annealing temperature (Tm), repeat motifs.

Primer	repeat	Start(bp)	End(bp)	Forword Primer (5'-3')	Tm(℃)	Reverse Primer (5'-3')	Tm(℃)
1	(A)10	4957	4966	CATAGCCGCACTTAAAAGCC	59.875	TGGGATCGTGCAATCAATTT	61.239
2	(A)10	12561	12570	CCATAAAGGAGCCGAATGAA	60.031	AGACAATGGACGCTGCTTTT	59.882
3	(A)10	40167	40176	ATCCCCTTCTCCATCGAAAT	59.728	AGCAAGATTGGTTGGATTGG	59.933
4	(T)10	76955	76964	GGGCAAATTATGTCAGTGCC	60.339	AGGCTATCTCAAACTGCCGA	59.978

Table 4(on next page)

Genetic diversity parameters estimated on 134 *Zantedeschia* accessions

Note: Na = No. of Alleles Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%

Ne = No. of Effective Alleles I = Shannon's Information Index

Table 4. Genetic diversity parameters estimated on 134 *Zantedeschia* accessions

Parameter	Section Zantedeschia	Section Aestivae	total
Na	3.000	14.000	14.000
Na Freq. >= 5%	3.000	6.500	7.000
No. Private Alleles	0.000	11.000	14.000
Ne	2.295	6.084	6.307
I	0.844	2.116	2.130

Note: Na = No. of Alleles

Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%

Ne = No. of Effective Alleles

I = Shannon's Information Index